AVIAN DISEASES 54:1108–1111, 2010

Case Report—

A Chronicle of Serologic Response in Commercial Layer Chickens to Vaccination with Commercial F Strain *Mycoplasma gallisepticum* Vaccine

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Received 30 November 2009; Accepted and published ahead of print 18 April 2010

SUMMARY. Vaccination of multi-age layer operations, wherein one million plus commercial layer chickens are housed, has been spurious until the development of a self-propelled, constant-speed spray vaccinator. Still, even with its use, live *Mycoplasma gallisepticum* (MG) vaccinations have been questionable in terms of seroconversion. Using the vaccinator as a research tool over the past 5 yr, factors have been elucidated which impact seroconversion to one live MG vaccine in particular, the F strain of MG (FMG). These factors include the type of nozzle used to spray the vaccine, the temperature of the water used to rehydrate and administer the vaccine, and the pH and osmolarity of the fluid used to apply the vaccine. In the present study, one farm was monitored for its seroconversion rates over 4½ yr, during which time the FMG vaccination protocol was amended as factors were identified that enhanced seroconversion rates. The results of this study showed that implementation and inclusion of the optimized factors into the vaccination protocol for FMG enhanced seroconversion rates because they went from an initial 50%–55% positive seroconversion rate to a consistent 100% positive seroconversion rate over the 56-mo study period.

RESUMEN. *Reporte de Caso*—Seguimiento de la respuesta serológica en aves de postura comerciales a la vacunación con una vacuna comercial de *Mycoplasma gallisepticum* cepa F.

La vacunación en las compañías de gallinas de postura comerciales de edades múltiples donde más de un millón de aves comerciales son alojadas, no ha sido efectiva hasta que se desarrolló un sistema de vacunación por aerosol con propulsión propia y con velocidad constante. Sin embargo, incluso con su uso, la vacunación con una vacuna viva de *Mycoplasma gallisepticum* (MG) han sido cuestionable en términos de la seroconversión. Mediante el uso de este sistema de vacunación como una herramienta de investigación durante los últimos 5 años, se han dilucidado los factores que tienen impacto sobre la seroconversión para una vacuna viva contra *M. gallisepticum*, en particular, contra la cepa F. Estos factores incluyen el tipo de boquilla utilizada para aplicar la vacuna en aerosol, la temperatura del agua usada para reconstituir y administrar la vacuna, el pH y la osmolaridad del líquido utilizado para aplicar la vacuna. En el presente estudio, se le dio seguimiento por cuatro años y medio a la tasa de seroconversión en una granja, durante ese tiempo, se modificó el protocolo de vacunación con la cepa F de acuerdo a como se fueron identificando los factores que aumentaban las tasas de seroconversión. Los resultados de este estudio mostraron que la aplicación y la inclusión dentro del protocolo de vacunación con la cepa F de dichos factores en condiciones óptimas, aumentaron las tasas de seroconversión contra la cepa F ya que las tasas que inicialmente eran del 50% al 55% pasaron a ser constantes en un 100% en el periodo de estudio de 56 meses.

Key words: chicken, layers, *Mycoplasma*, poultry, vaccination, disease

Abbreviations: FMG = F strain *Mycoplasma gallisepticum*; MG = *Mycoplasma gallisepticum*; PBS = phosphate-buffered saline; psi = pounds per square inch; PV = postvaccination; SPA = serum plate agglutination; WOA = weeks of age

In the United States, turkey and chicken primary and multiplier breeders and hatcheries generally have adopted the various *Mycoplasma gallisepticum* (MG) control programs of the National Poultry Improvement Plan (15). Initial efforts by the poultry industry to control and contain MG included testing and slaughter of reactor flocks. Ultimately, using the aforementioned measures coupled with heat treatment of hatching eggs (21), together with biosecurity and biosurveillance procedures (9), the entirety of the poultry breeder industry was cleared of MG. Owing to management in both the commercial broiler and turkey sectors, wherein "all-in, all-out" rearing practices were utilized, these two sectors have been able to maintain MG-free commercial flocks, save for the sporadic mycoplasmal outbreak. While all three sectors of the industry have experienced tremendous growth since the control efforts directed at MG were initiated, it is the table egg sector which has, arguably, experienced the most change in terms of management practices that have impeded maintenance of MG-free status. Specifically, it is the advent and use of multi-age production complexes, wherein 14 or more houses not only share a common environment but are also interconnected via walkways and egg belts which transport the eggs to the complex-associated egg processing plant. In each of the houses, 75,000 or more hens are maintained from 20–100 weeks of age (WOA). Once chickens are placed in these layer complexes, individual houses will not be depopulated until the birds reach 100 WOA, and the complex itself will constantly have chickens present during its approximate 20- to 30-year production service life. Thus, the presence of 1.5 million MG-free hens in a modern layer complex represents a population at risk. Complex managers have opted to make live MG vaccines an integral management practice in modern layer complexes because of inherent egg production and mortality losses, the cost of therapeutics associated with MG infection of hens in production, and the likely probability of MG gaining access to a layer complex at some point during the facility's service life.

The first of three currently commercially available live MG vaccines, FVAX-MG® (Schering-Plough Animal Health Corp.,

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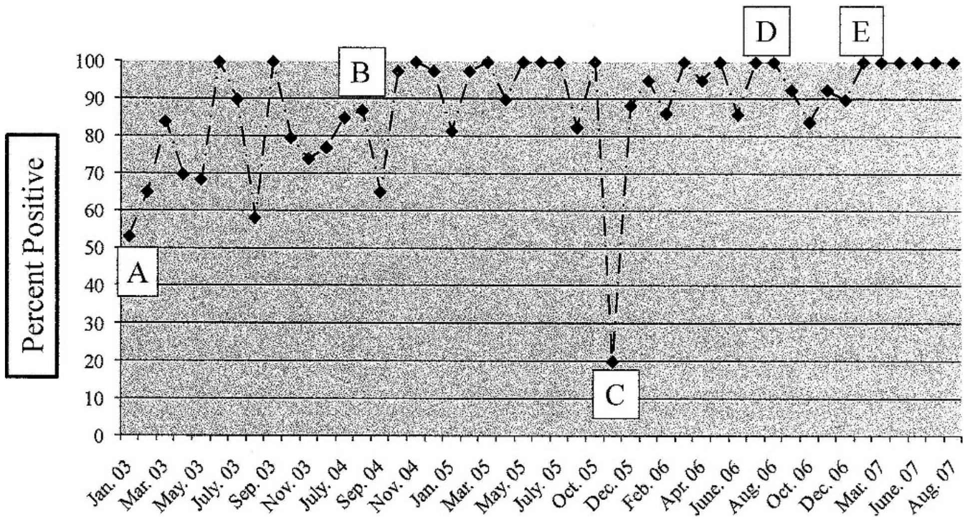

Fig. 1. SPA serologic response obtained from blood drawn 6-wk PV (FMG vaccine) from successive pullet flocks over a 56-mo period on a single commercial layer complex; the chronologic and cumulative inclusion of identified optimized parameters were included in the vaccination protocol. Points A–E represent the following: (A) no vaccination parameters identified or optimized, (B) 40 psi and coarse spray nozzles arbitrarily selected and utilized, (C) vaccination by individual unfamiliar with the then-current but developing MG vaccination protocol, (D) inclusion of product to adjust pH and osmolarity, and (E) use of 4 C water for vaccine rehydration and administration.

Omaha, NE), was licensed by the USDA in 1988, but the use of live MG vaccines by the commercial table egg industry predates USDA licensure. Early efforts included the administration of live MG inoculum via drinking water (10) or via individual eyedrop inoculation (3). Two of the three commercially available, live MG vaccines currently in use, which are approved for spray and eyedrop application, are F-VAX-MG (Schering-Plough Animal Health Corp.) and MYCOVAC-L (Intervet Animal Health, Millsboro, DE). The third live vaccine, MGTS-11 (Merial-Select, Gainesville, GA), is only approved for eyedrop administration.

Although spray administration often results in nonuniform and inconsistent seroconversion because some birds are profusely sprayed while others receive little or no vaccine, it is desirable for both ease and cost of application (2). While spray vaccination is the preferred method for inoculating the respiratory system of poultry, inconsistent or nonuniform administration can result in a "rolling reaction" with a flock, which in turn can result in longer recovery, with an overall impact of decreased flock performance. Within the commercial table egg sector of the poultry industry, spray application is considered to be the most effective delivery system for the administration of most MG vaccines (6). The development of a self-propelled, constant-speed spray vaccinator for use in caged layer chicken facilities has provided a means to study and optimize parameters such as water temperature, ionicity, and pH, as well as characteristics of pressure and nozzle type for applying live MG vaccines to layer chickens.

This report describes the serum plate agglutination (SPA) serologic response obtained from blood taken 6 wk postvaccination (PV) from successive pullet flocks over a 56-mo period on a commercial layer complex, wherein the chronologic and cumulative inclusion of specific optimized parameters were included in the live MG vaccination protocol.

CASE REPORT

Case description. All chickens were of a single strain (Hy-Line W-36), housed on a commercial layer complex, and were MG vaccinated with F-VAX-MG (Schering-Plough Animal Health Corp.) as pullets at 9 WOA throughout the 56-mo test period using the self-propelled, constant-speed vaccinator as previously detailed (6). As this was a working commercial table egg complex, no attempt was made to determine titer of any of the vaccine batches used over the 56-mo interval. All vaccine was stored as specified by the manufacturer and no vaccine was used beyond its expiration date. At 6 wk PV, 20 chickens were randomly selected from the vaccinated pullet house as previously described (6), and approximately 3 ml of blood were removed. Sera were obtained from each sample. SPA tests were conducted as described by Yoder (22), and commercial agglutination antigen (NOBILIS, Intervet Animal Health) was used with multiple, albeit unrecorded, lots or batches of antigen utilized throughout the course of the 56-mo study. Management of the layer complex was solely interested in 6-wk PV SPA tests results; no other serologic tests were conducted.

In early 2003, before any vaccination parameters were identified, the MG SPA response varied from 55% to 85% (Fig. 1, point A), and with the initial parameter of pressure set at 40 pounds per square inch (psi; based upon preliminary research, data not shown), the MG SPA response range through mid-2004 ranged from 55% to 100% positive (Fig. 1, point B). In September 2004, initial research at our laboratory showed that a 1× concentration of phosphate-buffered saline (PBS) prevented the *in vitro* two- to fourfold decrease in MG viability seen when live MG vaccines were diluted with water (distilled) alone (11). This observation was included as a defined parameter, and a commercial product (PBS, 1× Powder Concentrate [PBS Concentrate], Fisher Scientific, Fairlawn, NJ) was identified and included in the vaccine preparation protocol. This product not only made the distilled water isotonic, but also raised the pH of distilled water to 7.3–7.5; not the optimal pH of 7.8 identified for growth of mycoplasma (13), although closer than the 4.10–9.07 pH range recorded from over 40 samples of distilled water used to rehydrate and spray live MG vaccine to layer chickens (data not shown). Inclusion of the PBS-concentrate product into the MG vaccination protocol, together with the use of 40 psi to apply live MG vaccine, resulted in subsequent 6-wk PV MG SPA serologic responses which ranged from 65% to 100% positive, with but a single exception of 20% (Fig. 1, point C).

A careful review of the vaccination event, performed 6 wk prior to blood collection from the flock that evidenced the 20% positives, showed that the individual responsible for vaccination had been called away to active military duty. In his absence, another individual, although familiar with pullet vaccination overall but unfamiliar with preparation of the water used in live MG vaccine application, had vaccinated the chickens. The individual was informed as to the new protocol, and subsequent MG SPA serologic responses through July 2006 ranged from 85% to 100% positives (Fig. 1, point D).

In March 2007, the effect of water temperature used for both live MG vaccine rehydration and spray application was addressed. This research was pursued because it was noted that water used to rehydrate MG vaccine in the field reflected seasonal ambient temperatures, which varied from 1.7 C in the winter to 32.2 C in the summer. Results of that study showed that the F-VAX-MG vaccine retained viability longer, and with higher titers, when 4 C water was used (5). This finding was included in the MG vaccination protocol. Inclusion of this parameter, together with the aforementioned optimized parameters, resulted in a uniform 100% MG SPA seroconversion rate (Fig. 1, point E) through the remainder of the study period, ending August 2007.

Like the preliminary selection of 40 psi for vaccine spray application, the initial selection of coarse spray nozzles (Model 806-09, K-15, Intervet Animal Health) for use in vaccine application was entirely arbitrary, although serendipitous. Subsequent to identification and incorporation of optimal water temperature, osmolarity, and pH parameters into the vaccination protocol, research was initiated into both the pressure: 310.2 (40 psi) versus 448.1 (60 psi) kPa utilized to dispense the vaccine, and into three different spray nozzles which included 1553-10 (defined as coarse by supplier), 1553-08 (defined as medium by the supplier), and 1531-06 (defined as fine by the supplier; HARDI, Taastrup, Denmark). Results by Purswell *et al.* (16) showed that very few respirable droplets (<10 μm) were observed for any treatment. Coverage and deposition were highly varied between the nozzle types, but vaccine viability was unaffected by any of the treatments tested. Droplet sizes were relatively similar between the nozzle types, but disparity was evidenced for both coverage and deposition between nozzle types (both parameters greatest for the 1553-10 nozzle), which indicated that coverage and deposition is of greater concern than droplet size in application of F-VAX-MG vaccine.

DISCUSSION

Interest in MG vaccines originated in the late 1970s as it became apparent that MG infection was endemic in some multiple-age, egg-laying complexes (14). The ability of mycoplasma to immunomodulate host immune responsiveness contributes to their pathogenic properties (19); yet they are also the smallest and simplest self-replicating microorganisms (1) and are extremely fastidious with regard to their nutrient–growth requirements in the laboratory (4). While the parameters for *in vitro* growth are fairly well defined, it is the administration of the organism as a vaccine that can effect the viability of the organism before confronting the host's immune system. This is particularly true for a spray-applied vaccine in the field, under the day-to-day changing environmental and personnel conditions in which the commercial table egg layer industry operates; such conditions introduce other factors that are less well known and, arguably, less controllable.

While the mycoplasma's total lack of a cell wall is the single, most-important characteristic that distinguishes them from other prokaryotes (18), it is this same physical characteristic that is believed to be the primary contributor to the organism's relatively poor viability outside its host. The cell membrane of MG is 110–112 A in thickness (7), and it is the presence of a cell membrane, as opposed to a cell wall, which makes the organism more sensitive to osmotic shock (17) and, ultimately, to cell death. Similarly, the lack of a cell wall makes the MG organism more susceptible to death from ultraviolet irradiation and dessication. As serologic responses to the mycoplasmas in general, and to MG in particular, are highly dose dependent (8,9,12,20), mycoplasmal death which occurs between rehydration of the vaccine and administration to the chicken equates to reduced titer.

It was originally theorized that the development and use of a self-propelled, constant-speed vaccinator in administering live MG vaccine to commercial layer chickens would decrease variation of 6-wk PV seroconversion rates. However, other factors have been demonstrated to be of importance in effecting improvement in 6-wk PV seroconversion rates. The source of water, together with the varying mineral concentrations, pH, ionicity (11), and the range of water temperatures experienced in the annual course of MG vaccine rehydration and application (5), in addition to the vaccinator spray nozzles used by the commercial table egg industry, are but a few factors which have been shown to impact viability, seroconversion, or both to MG. As each factor that impacts seroconversion is discerned, defined, and optimized, more uniform growth and performance—together with enhanced health status—should be increasingly evident. Indeed, the data from the present study show incremental improvement in 6-wk PV seroconversion rates as each optimized parameter was included in the live MG vaccination protocol. Although the particular complex chronicled herein demonstrates 100% SPA rates during the final 6 mo of the study, it is also recognized that other factors very likely exist that could be optimized to further enhance seroconversion rates across all complexes that utilize live MG vaccines. Indeed, perhaps the utilization of other serologic tests could provide additional insight regarding either the parameters thus far identified, or others that remain to be discerned, that significantly impact seroconversion. In the interim, the serologic results derived from incorporation of the aforementioned optimized parameters, into a F-VAX-MG vaccine application protocol in layer chickens, are perhaps summed by stating that some parameters appear to more important than others; even so, inclusion of each into a vaccination protocol is important to both rapid and comprehensive serologic conversion.

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